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Complex Coacervates for Thermally Sensitive Controlled Release of Flavor Compounds

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To improve the appeal of frozen baked foods upon heating, we have encapsulated flavor oil in complex coacervate microcapsules using gelatin and gum Arabic. Variation of polyion concentrations and homogenization rate affected particle morphology, size distribution, and oil release upon heating. Release of the oil from formulations was determined by a simple spectroscopic method based on separation of oil labeled with a lipophilic dye from unaffected particles. When heated to 100 °C or higher, univesicular microcapsules (prepared with a lower homogenization rate) released almost all of the encapsulated oil, while multivesicular microcapsules (produced by high homogenization rates) resulted had lesser degrees of release. The oil remained encapsulated during 4 weeks of storage at 4 and −20 °C (freezing and thawing) but was released by exposure to 100 mM NaCl at room temperature. When particles were cooled after releasing their oil content, the oil was re-encapsulated.

KEYWORDS: Complex coacervation; gelatin-gum Arabic; flavor oil; frozen foods; heat-triggered release; controlled delivery

INTRODUCTION

The flavor of baked goods can constitute a significant component of their appeal. The smell of breads, pastries, and pizzas at their sites of manufacture (bakeries and pizzerias), where large quantities of the product are made in a confined space, has a considerable appetitive effect. Unfortunately, this feature is generally diminished or lacking in frozen preparation, particularly in those designed for microwave cooking.

Here, we describe a methodology whereby the flavor of such foods can be enhanced. The design criteria of the system are (1) that the odor should not be released while the food is frozen or, if thawed, before it is cooked, (2) that the odor should be released upon heating, and (3) that the controlled release system be (a) stable frozen for extended periods of time (i.e., the release mechanism should not shorten the frozen shelf life of the unmodified food), (b) easily applicable in bulk to the food, without altering the basic production method, (c) composed of acceptable food-grade material, (d) low in cost (given the low-margin/large scale nature of the industry), and (e) easily scalable in manufacturing. In some cases, it is also important that the system be able to withstand the aqueous phase of the food. (For example, in pizza, the aqueous phase has a pH of approximately 4. The salt concentration varies considerably between tomato pastes/sauces.) In addition, it would be desirable for the heat-triggered release of flavor to recur on subsequent reuse of the food (e.g., reheating after interval refrigeration).

Microencapsulation techniques have widely been used for a variety of food applications such as taste and odor masking (*1*), prolonging organoleptic effects of flavor or other sensory markers (*2*), protection of food ingredients that are chemically unstable under storage or cooking conditions (temperature, moisture, oxygen, etc.) (*3*, *4*), or developing functional microcapsules that release flavoring ingredients under specific circumstances [for example, upon chewing (*3*), under elevated temperature and low-moisture conditions (*5*) or under low-temperature and high-moisture conditions (*6*)]. Various microencapsulation technologies such as spray drying, fluidized bed, coacervation, liposomes, and supercritical-fluid-based techniques have been employed for encapsulation of food ingredients with ever-increasing popularity (*7*).

To develop a microcapsule system that satisfies the aforementioned requirements, we have used a complex coacervation microcapsule system (*8*, *9*) to encapsulate a flavor oil in a complex of two hydrophilic polymers. Neutralization of the positive charge of one of the polymers by the negative charge of the other causes phase separation of the polymer-rich phase. Specifically, we used the gelatin-gum Arabic (GGA) system (*10*), which has been used for various consumer products including carbonless copy paper (*11*), scent strips, fragrance

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Table 1. Microencapsulation Conditions

homogenization rate (rpm)	concentrations of gelatin and gum Arabic solutions (%)	average volume mean diameter (μm) ($n = 3$)
3000	2	82.7 ± 3.7
9000	0.5	not measured
9000	1	92.0 ± 4.1
9000	2	67.1 ± 9.2

samplers, and flavor ingredients (*3*, *12*) and is an investigational method for microencapsulating lipophilic drugs (*13*, *14*).

The GGA method suited this application because (1) the solubility of the capsule wall is temperature-dependent because of the gelatin; and (2) the ingredients are nontoxic, nonirritating, and available in food grade and have been widely used as food additives and in a variety of oral pharmaceutical formulations (*15*). Consequently, the formed microcapsules can be used without exhaustive purification processes.

MATERIALS AND METHODS

Materials. Gelatin (type B from bovine skin, gel strength 60 Bloom), gum Arabic (Acacia senegal, from Acacia tree), Nile Red, and 1-octanol were purchased from Sigma−Aldrich (St. Louis, MO). Glacial acetic acid was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Bake flavor oil (Natural Spice Flavor for Baked Foods, 10% natural oils from anise, oregano, garlic, and black pepper; 90% vegetable oil) was a gift from Kraft Foods, Inc. (Glenview, IL).

Microencapsulation Using the Complex Coacervation Method. Solutions of gum Arabic and gelatin of varying concentrations were prepared (**Table 1**). For gelatin solutions, a water bath of 50 °C was used to facilitate dissolution of the gelatin. Bake flavor oil (1.125 mL) optionally containing 1 mg/mL Nile Red was homogenized in 25 mL of gelatin solution at specified rates using a Silverson homogenizer (L4RT-A, East Longmeadow, MA). Here, Nile Red was added to the oil to enhance visual detection as well as quantitative determination of the encapsulated oil. Gum Arabic solution (25 mL) was added dropwise into the oil emulsion in the gelatin solution, maintaining the temperature at 50 °C. The pH of the entire mixture was adjusted to 4 using 0.1 M acetic acid. Then, the mixture was stirred in an ice bath for 20 min until it reached a temperature of 4 °C. The microcapsules sank to the bottom of the aqueous phase as they formed. After excessive supernatant was decanted, the microcapsule suspensions were divided into 1 mL aliquots, which were then used in studies.

Morphology and Size. Microcapsules were visualized by brightfield microscopy (Axiovert 200, Zeiss, Thornwood, NY). The size distribution was determined by microscopy and with a Beckman Multisizer3 Coulter Counter (Fullerton, CA) using an aperture size of 400 μm.

Gas Chromatograph/Mass Spetctrometer (GC/MS) Analysis of the Original and Encapsulated Oil. The original and encapsulated oil were analyzed using an Agilent 5973N GC/MS. The microcapsule suspension (prepared with 1% gum Arabic and gelatin solutions at 9000 rpm) were in 200 mM NaCl for 30 min to dissolve the microcapsule walls and release the encapsulated oil. An equal volume of dichloromethane was then added and mixed with the suspension by mechanical agitation for 1 min. The encapsulated oil extracted into the dichloromethane was analyzed with GC/MS, and the chromatographs were compared with those of the original oil that underwent the same extraction procedure. A total of 1 μL of each sample was injected into the column (Restek Rtx-1, Crossbond 100% dimethylypolysiloxane, 30 m × 250 μm × 1.00 μm) that was programmed for a 5 min solvent delay at 100 °C, followed by heating to 250 °C at a rate of 20 °C/min (1 °C/min from 150 to 160 °C), holding at 250 °C for 5 min, and then heating to 320 °C at a rate of 30 °C/min. Helium was used as a carrier gas and flowed at 1 mL/min.

Release Kinetics. Aliquots (1 mL) of microcapsule suspensions were left at room-temperature overnight and then exposed to experimental conditions: high temperatures (100 and 200 °C), NaCl solutions (50 or 100 mM for 1 or 24 h), 200 mM sucrose solutions for 1 or 24 h, and storage temperatures (4 and −20 °C) for varying periods (overnight and 4 weeks). One group of microcapsules was heated to 100 °C for 30 s and then cooled in an ice bath for 30 min before measuring oil release. Control groups did not undergo those experimental conditions but were processed for determination of oil release after being at room-temperature overnight. For all groups, the extent of oil release was measured as follows.

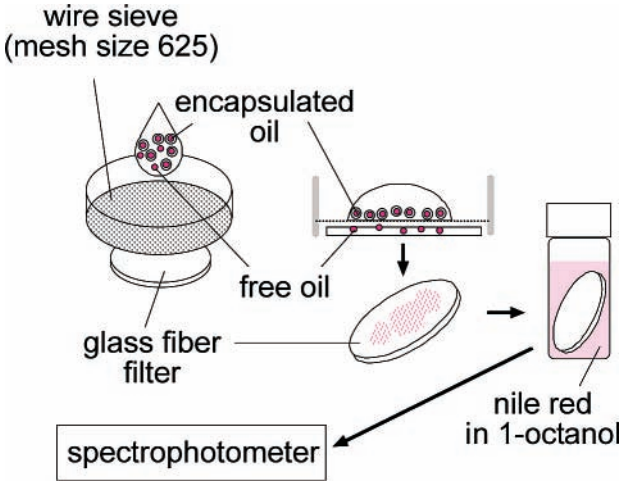

Figure 1. Schematic of measuring the released bake flavor oil.

Determination of the Released Oil (Figure 1). The encapsulated oil contained Nile Red, a lipophilic dye. Particles in aqueous suspension were exposed to the above experimental conditions and then poured onto a wire mesh (mesh size 635, opening size of 20 μm, Newark wire cloth company, Newark, NJ). The released oil was separated from the microcapsules (and unreleased oil) by rinsing with water. The rinsate was recovered on three to five disks of glass fiber filters (Whatman Grade GF/B, 2.5 mm diameter). The dye was extracted from the water in 5 mL of 1-octanol with continuous shaking for 96 h (1-octanol was chosen as an extraction medium because it readily dissolves Nile Red but is not miscible with water; therefore, its volume remains the same irrespective of the volume of added water). The absorbance of Nile Red in 1-octanol was measured at 550 nm with a microplate reader (Molecular Devices SpectraMax 384 plus) and compared to standard curves prepared with known concentrations of Nile Red (originally dissolved in the oil at 1 mg/mL) diluted with 1-octanol. The standard curves displayed a high degree of correlation ($r^2 = 0.9994$) between the absorbance and concentration.

Statistical Analysis. Because the data from release studies were not normally distributed, numeric data were expressed and charted as medians with 25th and 75th percentiles. Statistical inferences were made using Mann−Whitney U-tests and/or Kruskal−Wallis tests using SPSS software (Chicago, IL). A p value < 0.05 on a two-tailed test was considered statistically significant.

RESULTS

Formation of Microcapsules. Microcapsules were successfully formed using all conditions listed in **Table 1**. The bake flavor oil provided by Kraft is intensely aromatic, but the odor decreased dramatically (to the subjective perception of the experimenters) when the gum Arabic was added. Furthermore, upon formation of microcapsules, the totality (to visual inspection) of the colored oil, which otherwise would have floated on the surface of water, settled at the bottom of the container. These observations indicate that the oil was efficiently encapsulated.

Particle morphology depended on the homogenization rate and the concentrations of gelatin and gum Arabic solutions (**Figure 2**). Low homogenization rates produced large univesicular microcapsules containing a single large oil core (**Figure 2A**), while high rates produced large multivesicular microcapsules containing many small oil cores within a polymeric shell.

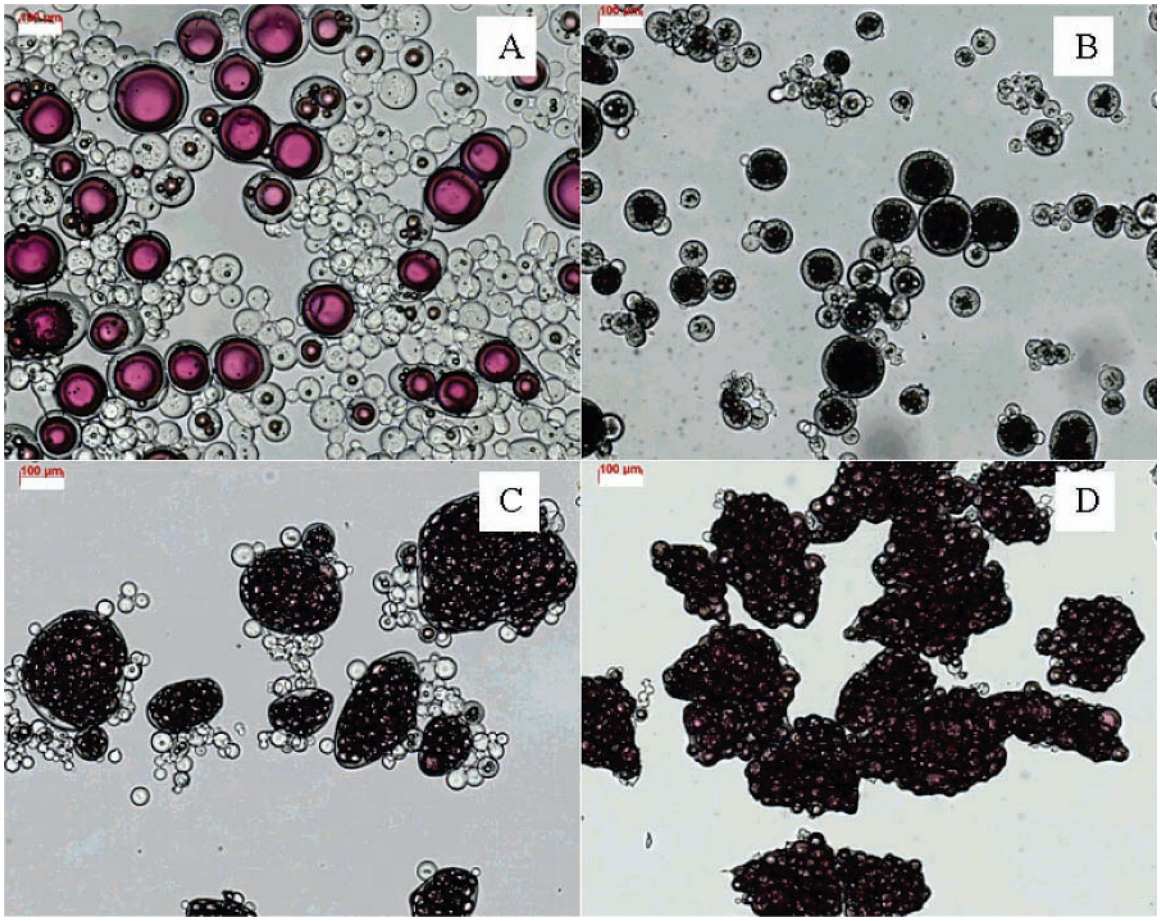

Figure 2. Microcapsules observed by a bright-field microscope: (A) homogenization at 3000 rpm, 2% polymer concentration; (B) homogenization at 9000 rpm, 2% polymer concentration; (C) homogenization at 9000 rpm, 1% polymer concentration; (D) homogenization at 9000 rpm, 0.5% polymer concentration. All scale bars = 100 μm.

To visual inspection under microscopy, when a lower concentration of polyions was used, the external coatings surrounding small oil cores were thinner (parts **C** and **D** of **Figure 2**) in comparison to those shown in parts **A** and **B** of **Figure 2**, and the size of the clusters or aggregates of cores increased. Measurements of particle/aggregate size using a Coulter counter also showed an increase in size with decreasing polymer concentration (**Table 1**). However, those data do not take into account the microcapsules that involved aggregates of a large number of oil droplets (e.g., those shown in **Figure 2**), which were larger than the orifice size used with the Coulter counter. In fact, the microcapsules prepared with the most dilute polymer solutions (0.5%) formed a significant amount of aggregates, and the size was not reliably measured with the Coulter counter. Reducing the concentrations of gelatin and gum Arabic solutions also decreased the frequency of free coacervates (i.e., polyion particles with no oil core). The rate of cooling of the emulsion did not significantly influence the formation of free coacervates (data not shown). Variation of the homogenization rate did not bring about a significant difference in particle size distribution.

Gas Chromatograph/Mass Spectrometer (GC/MS) Analysis of the Encapsulated Oil. The chemical integrity of the encapsulated oil was verified using GC/MS analysis. As shown in **Figure 3**, the total ion chromatograms (**Figure 3A**) and mass spectra of each peak (two representative peaks are shown in **Figure 3B**) were identical. The encapsulated oil was chemically identical to the original flavor oil, which indicated that the encapsulation process did not cause significant loss of any component of the original oil.

Development of the Method for Quantitating Oil Release. To study release kinetics, we developed a method to separate oil released from disrupted particles from oil that was still encapsulated (see the Materials and Methods). Parts **A** and **C** of **Figure 4** show that microcapsules retained on the wire mesh (i.e., those not disrupted by experimental conditions) were morphologically similar to the untreated microcapsules shown in parts **A** and **B** of **Figure 2**. Liquid collected under the wire mesh after heating at 100 °C for 30 s was composed of either a mixture of free oil in a dilute aqueous solution of polyions (thin arrow in parts **B** and **D** of **Figure 4**) or oil droplets within the polymer residue (thick arrow in **Figure 4D**). It is likely that the polymer shells (**Figure 4D**) were the results of recoacervation of the polyions (which passed through the mesh after dissolving at high temperature) upon cooling, because the faint color of the oil compared to that in encapsulated oil droplets suggests a flattened two-dimensional structure and the particle size in **Figure 4D** is larger than could pass through the mesh. Nile Red was completely extracted from the glass fiber filter to 1-octanol after immersion in that solvent in 96 h.

Heat Responsive Release Kinetics. Particles were maintained in suspension at room temperature overnight and then received either no further treatment or their container was left in a heating block at 100 or 200 °C, and oil release was measured ($n = 4$). There was no significant difference in oil release from the four formulations tested in the absence of heating ($p = 0.35$ by Kruskall−Wallis test; **Figure 5**). Heating resulted in a dramatic increase in the oil release for all formulations ($p = 0.029$ for all comparisons of the negative control to corresponding heated

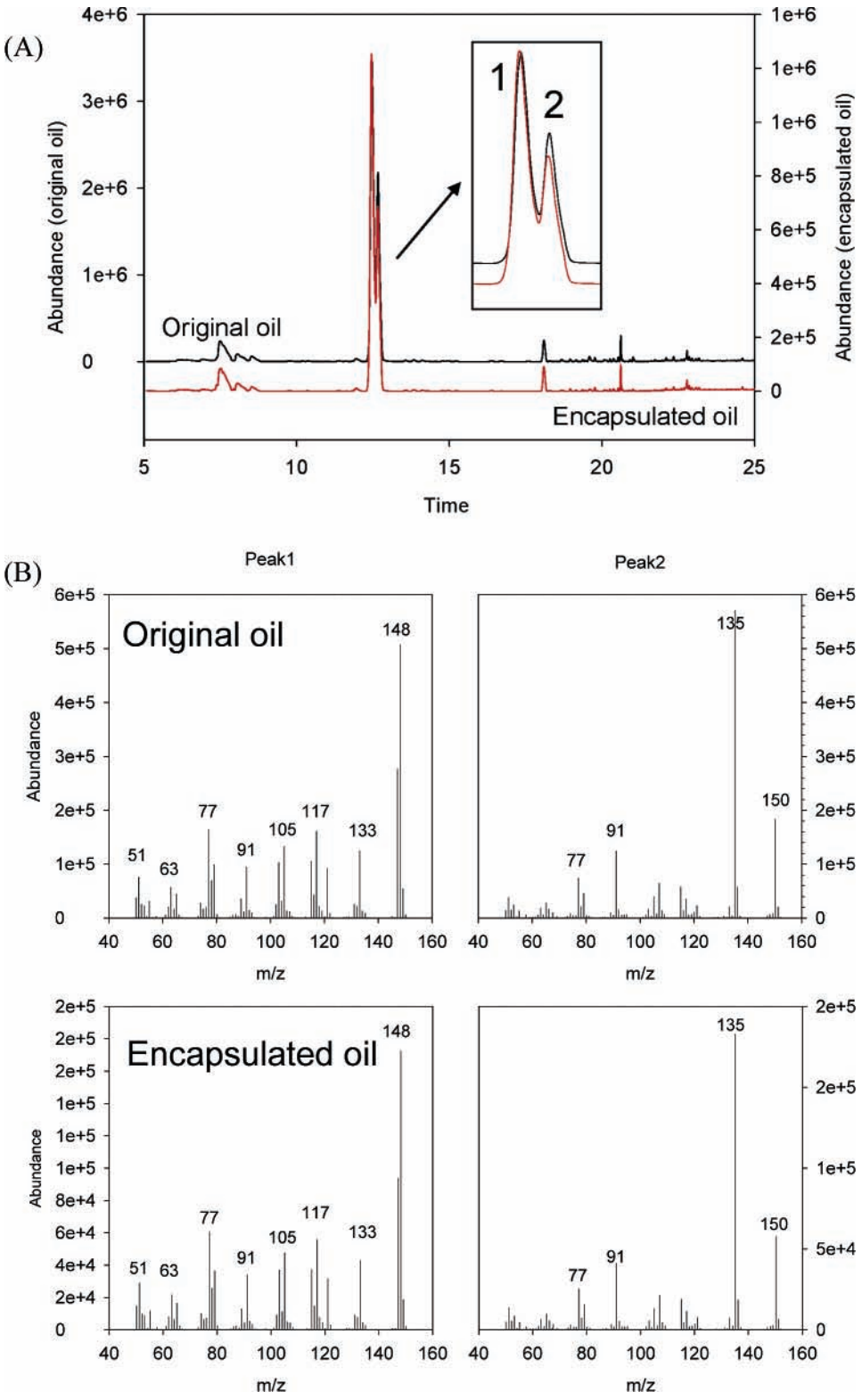

Figure 3. Comparison of original and encapsulated oils: (A) total ion chromatograms and (B) mass spectra of peak 1 and peak 2.

samples). Release at 200 °C was no greater than at 100 °C. Microparticles formed by homogenization at 3000 rpm had a greater release in response to heat than the same composition of matter homogenized at 9000 rpm ($p = 0.029$). Oil release increased with increasing polyion solution concentrations (e.g., when the amounts of oil release were compared by the three formulations made at 9000 rpm at 100 °C, the p value from the Kruskal−Wallis test = 0.018, and at 200 °C, $p = 0.01$).

To assess whether this system could be recycled, we studied particles that were heated then cooled in an ice bath. It was observed by microscopy that the capsule walls that were destroyed by heating reformed upon cooling (**Figure 6**). The median percentage of released oil was 92.9% (25th percentile, 86.9%; 75th percentile, 96.1%) when measured shortly after heating at 100 °C but decreased ($p = 0.034$) to 10.4% (25th percentile, 8.8%; 75th percentile, 12.6%) after cooling, which

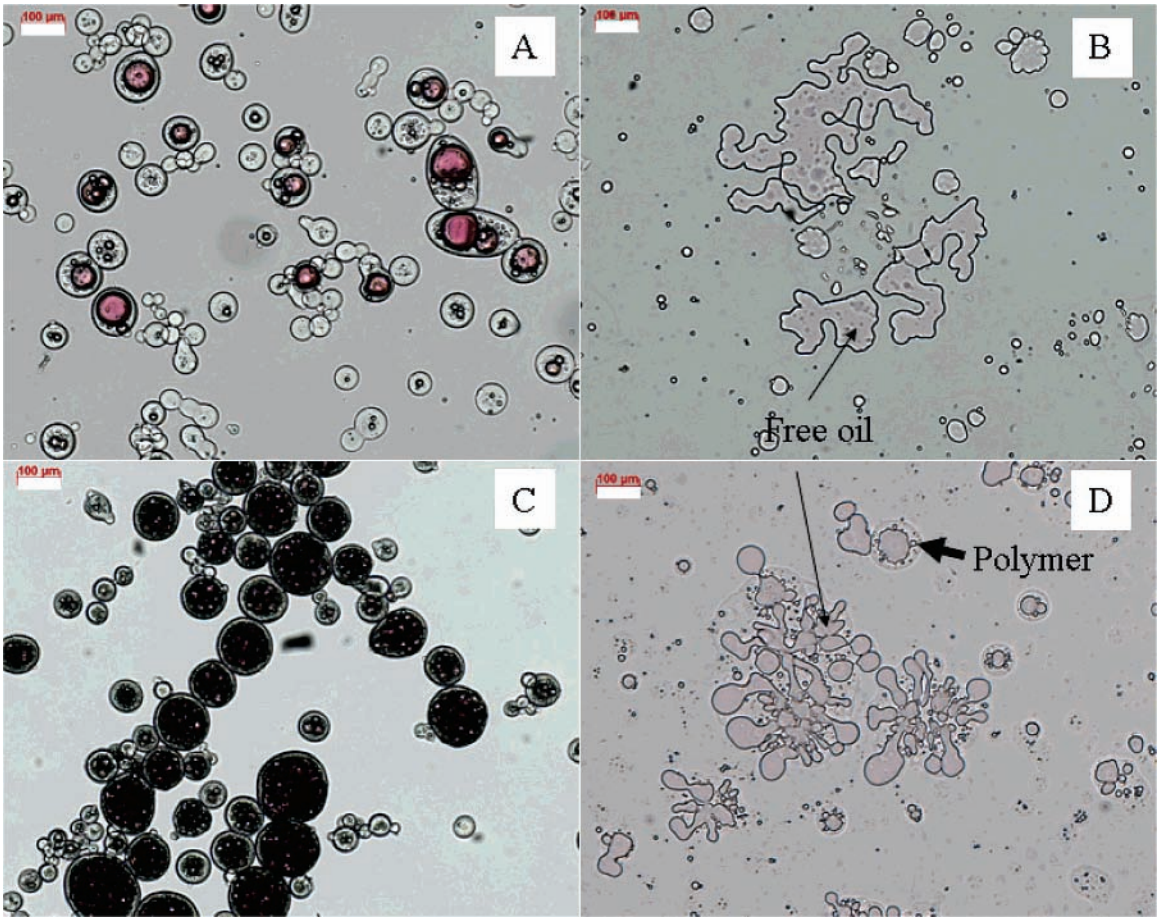

Figure 4. Microcapsules recovered after being retained on the mesh (A and C) and free oil passing through the mesh (B and D). (A) Homogenization rate 3000 rpm, 2% polymer solution. (C) Homogenization rate 9000 rpm, 2% polymer solution. (B) Free oil separated from A. (D) Free oil separated from C. Thin arrow = free oil. Thick arrow = polymer shell around oil. All scale bars = 100 μm.

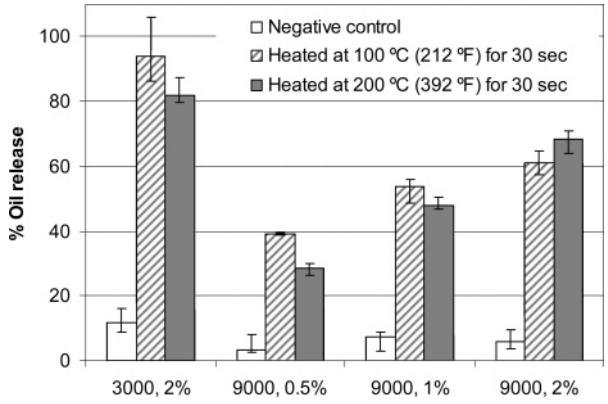

Figure 5. Heat responsive release of the oil released from GGA microcapsules. Data are medians with 25th and 75th percentile bars (n = 4). All groups were maintained at room temperature overnight and then given the treatment indicated. Negative control = no further treatment.

was comparable to release from unheated controls. There was a marked increase of capsule size, presumably because of the increased number of encapsulated oil droplets (**A** versus **B** in **Figure 6**). The particles shown in **Figure 6B** were formed when well-suspended particles were heated and then cooled. This phenomenon also occurred when particles were allowed to aggregate by settling prior to heating (parts **C** and **D** of **Figure 6**), a condition that may be a more accurate reflection of what might occur in a food product.

Stability of Microcapsules. Stability during storage and handling is an important consideration in the design of frozen foods. Microcapsule suspensions were maintained at room temperature overnight, then were stored at 4 or −20 °C, overnight or for 4 weeks, and then returned to room temperature. The free oil released during storage was then measured (**Figure 7**). For the 3000 rpm, 2% polymer and 9000 rpm, 0.5% polymer formulations, the Kruskal−Wallis test did not detect statistically significant differences between storage groups and untreated particles (i.e., particles maintained at room temperature overnight without further storage). In the other two formulations, there were statistically significant differences, but they were very small in magnitude. These results suggest that these uncross-linked microcapsules could be stored for at least 4 weeks with minimal or no loss of integrity.

To examine the effect of salinity on the physical stability of the microcapsules, the microcapsules were treated with 50 mM NaCl, 100 mM NaCl, or 200 mM sucrose for 1 or 24 h at room temperature (n = 4) (**Figure 8**). NaCl solution (50 mM) did not cause a statistically significant release of oil for 24 h (except for the single case of the 3000 rpm, 2% polymer formulation, where there was a modest increase in release, p = 0.029, but we note there was a high outlying value that affected the results). On the other hand, all four sets of microcapsules stored in 100 mM NaCl solution showed very large and statistically significant degrees of oil release in 1 h (p = 0.029 in all cases). Oil release was not significantly increased over the untreated group (control) upon exposure to equi-osmolar sucrose solution for the same period.

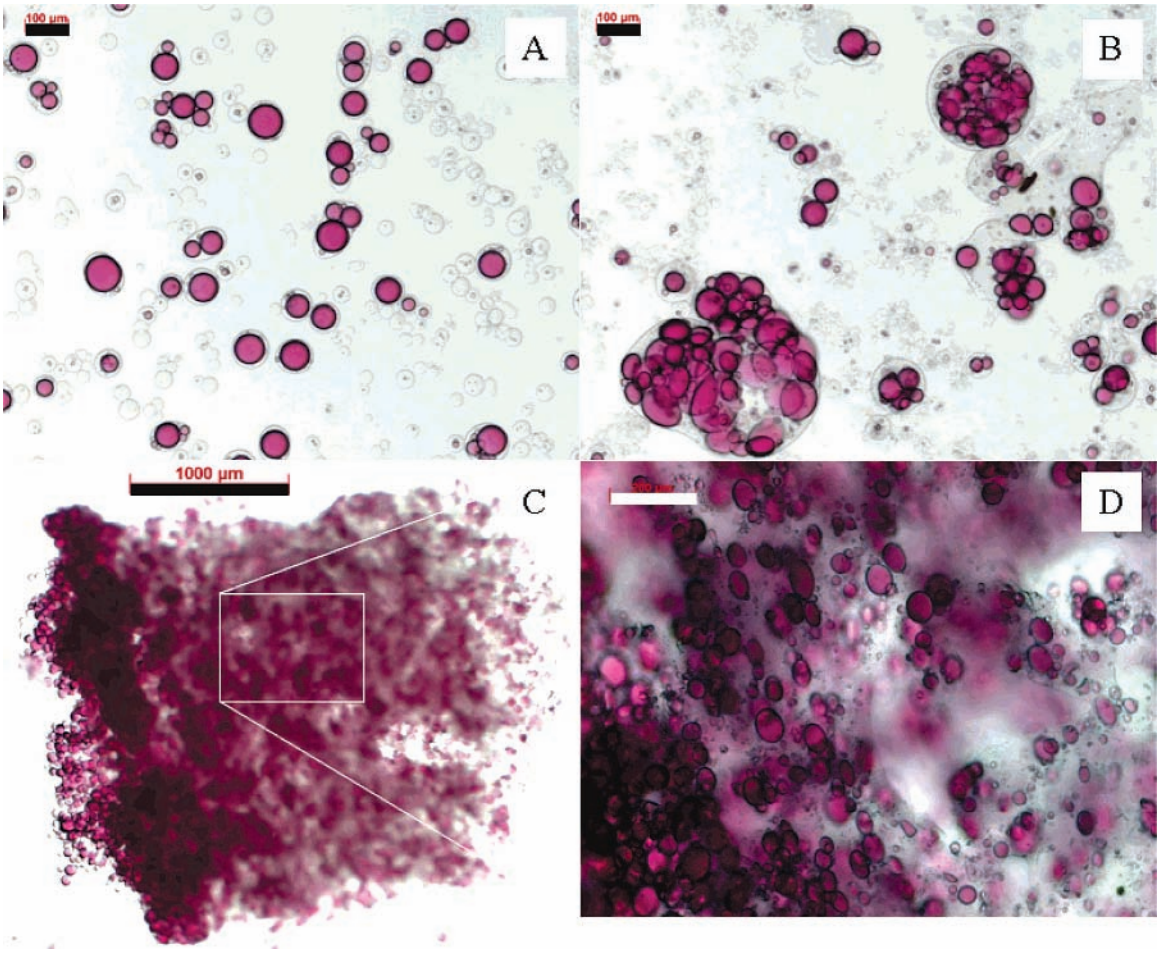

Figure 6. Recoacervation of heated microcapsules upon cooling: (A) original microcapsules, (B) microcapsules after heating followed by cooling, (C) low-power view of microcapsules that were allowed to settle prior to heating and cooling, (D) magnified view of C. Scale bars: (A) 100 μm, (B) 100 μm, (C) 1000 μm, and (D) 200 μm.

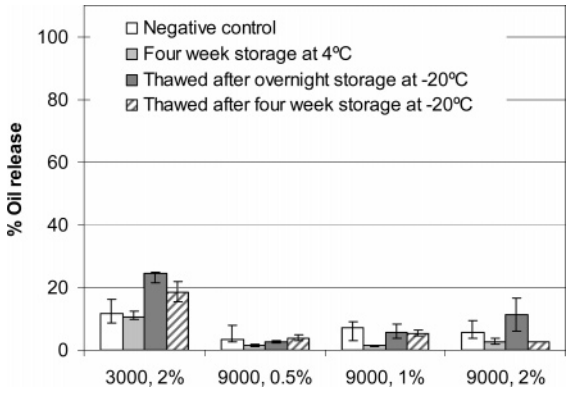

Figure 7. Oil release from GGA microcapsules upon storage at 4 and −20 °C for different periods. Data are medians with 25th and 75th percentile bars ($n = 4$).

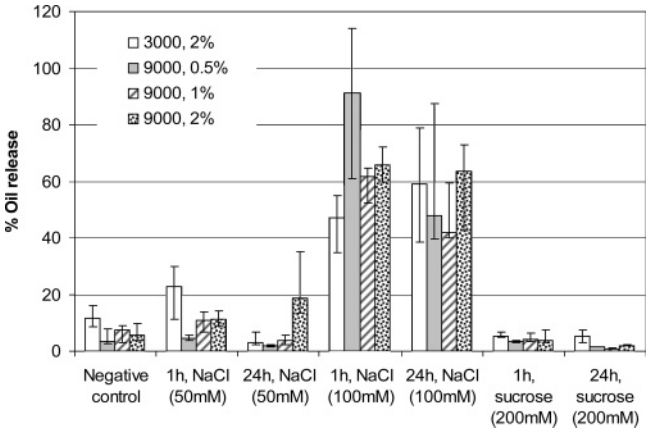

Figure 8. Oil release from GGA microcapsules upon exposure to sucrose and NaCl solutions. All groups were maintained at room temperature overnight and then given the treatment indicated. Negative control = no further treatment. Data are medians with 25th and 75th percentile bars ($n = 4$).

DISCUSSION

The microcapsule system employed here appears to meet the design criteria established in the Introduction and therefore could be used to provide temperature-dependent release of flavors from food products. It also had appropriate storage characteristics, although one would expect that the specific formulations developed here would release much of their flavor if kept at room temperature for extended periods of time in foods containing a high salt content. Furthermore, the reversible release behavior of this system suggests that the appetitive effects of the flavor burst could be sustained through repeated heating/freezing cycles.

Here, we produced microcapsules containing the bake flavor oil. The chemical integrity of encapsulated oil was confirmed by GC/MS analysis. We note that these complex coacervate microcapsules were produced and used without chemical cross-linking. Cross-linking agents such as glutaraldehyde or form-

aldehyde are inappropriate for pharmaceutical and food applications because of the potential toxicity, and it has been shown that microcapsules can be stable without chemical cross-linking (*8*, *13*). Furthermore, uncross-linked microcapsules might present advantages for the present application, where ready dissolution of the capsule wall upon heating is desirable. In addition, because all of the materials used here were of food grade materials, there was no need for further purification of the final product, which would incur additional processing and cost. Acetic acid was used here to adjust pH; we also found that hydrochloric acid could be used without affecting the formation of microcapsules (data not shown). Acetic acid could be replaced with other acids used as food additives (i.e., citric acid) should the potential flavor effects of acetic acid be a concern or should the use of an alternative acid favor a particular flavor formulation.

The morphology and size distribution of the microcapsules were affected by the concentration of the polyion solutions and the rate of homogenization in generating emulsions of the oil-gelatin solution. The rate of homogenization affected the size of oil cores encapsulated within the microcapsules, whereas the polyion concentrations affected the number of core aggregates consisting of a microcapsule. These morphological differences, in turn, influenced the stability of microcapsules under the various release conditions. The comparison between 3000 and 9000 rpm shows that the microcapsules prepared with a lower homogenization rate were less resistant to heating. A possible explanation for this result is that slower homogenization formed microcapsules having single large cores, which may make them more vulnerable to damage than multivesicular microcapsules produced by higher rate homogenization. Microcapsules prepared with higher concentrations of polyions were less resistant to the release conditions. This result, which is counterintuitive, may be due to the fact that lower solution concentrations resulted in bigger agglomerates of oil droplets, which were harder to break down completely.

There was a considerable degree of aggregation with some formulations. It is unlikely that this would present a problem for the application under consideration as it might, for example, in particles designed for biomedical use. The aggregates here are small enough to be easily delivered in a manufacturing plant.

Given that electrostatic interactions are critical in complex coacervation, the stability of the microcapsules might be influenced by the ionic strength of the environment. Commercial tomato pastes or purees contain 10−100 mM of various salts depending on the brand. Our stability tests using 50 and 100 mM NaCl solutions showed that the stability of microcapsules depended on the concentration of salt. On the other hand, the microcapsules maintained their stability in a sucrose solution with the same osmolarity as that of 100 mM NaCl, which suggests that the instability of the microcapsules in NaCl was not due to osmotic pressure but because of the disruption of electrostatic interactions between the two component polyions (gelatin and gum Arabic). While the data suggests that the integrity of the microcapsules would be compromised by prolonged exposure to high concentrations of salts, this is less likely to be of concern in the frozen foods, in which the salt diffusion process is expected to be slower than in the solution state at room temperature.

We developed the spectroscopic method of measuring oil release used here to provide an accurate assay that was neither as cumbersome nor as prone to error as some other tests. The encapsulation efficiency and the release behavior of encapsulated oils have been determined by gas chromatographic analysis of volatiles released into the headspace (*16*, *17*) or extracted into organic solvents (*18*), measuring the weight loss after evaporation of the flavor oil (*19*) or the volume of the oil recovered by distillation (*17*, *18*). We note that the method reported here measures the total release of the oil phase but does not distinguish between release due to particle rupture and release by diffusion through the hydrophilic particle shell. In this particular formulation, where all of the components are hydrophobic, it is unlikely that the latter is a significant contributor to total release. However, it could be a concern with components with significant solubility in water.

Supporting Information Available: A movie of heat-triggered release of the bake flavor oil upon adding hot water to the microcapsule suspension and the microcapsules burst release the colored oil into the surrounding water. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Andersen, S. Microencapsulated omega-3 fatty acids from marine sources. *Lipid Technol.* **1995**, *7*, 81−85.

(2) Shefer, A.; Shefer, S. D. Multicomponent controlled release system for oral care, food products, nutraceutical, and beverages. U.S. Patent 6,887,493, 2005.

(3) Wampler, D. J.; Soper, J. C.; Pearl, T. T. Method of flavoring and mechanically processing foods with polymer encapsulated flavor oils. U.S. Patent 5,759,599, 1998.

(4) Krumbholz, R.; Lamprecht, A.; Lehr, C.-M.; Schafer, U.; Schirra, N.; Treitz, M. Microencapsulated unsaturated fatty acid or fatty acid compound or mixture of fatty acids and/fatty acid compounds. U.S. Patent 6,234,464B1, 2001.

(5) Rotman, A.; Blatt, Y. Heat stabilized flavoring agents coated with hydrogenated caster oil. U.S. Patent 5,098,725, 1992.

(6) Merchant, Z. M.; Behringer, M. U.; Hill, L. G.; McPherson, A. E.; Tangprasertchai, U.; Vogt, R. A.; Nayyar, D. K. Low-temperature cooking cereal-based products. U.S. Patent 6,599,-550, 2003.

(7) Gouin, S. Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends Food Sci. Technol.* **2004**, *15*, 330−347.

(8) Burgess, D. J.; Singh, O. N. Spontaneous formation of small sized albumin/acacia coacervate particles. *J. Pharm. Pharmacol.* **1993**, *45*, 586−591.

(9) Singh, O. N.; Burgess, D. J. Characterization of albumin−alginic acid complex coacervation. *J. Pharm. Pharmacol.* **1989**, *41*, 670−673.

(10) de Jong, H. G. B.; Hoskam, E. G. Behavior of microscopic substances consisting of biocolloidal systems and suspended in aqueous media. VI. Composition of the hollow spheres formed of complex coacervate drops. *Proc. Acad. Sci. Amsterdam* **1942**, *45*, 200−203.

(11) Green, B. K.; Schleicher, L. Oil-containing microscopic capsules and method of making them. U.S. Patent 2,800,457, 1957.

(12) Soper, J. C. Method of encapsulating food or flavor particles using warm water fish gelatin, and capsules produced therefrom. U.S. Patent 5,603,952, 1997.

(13) Jizomoto, H.; Kanaoka, E.; Sugita, K.; Hirano, K. Gelatin-acacia microcapsules for trapping micro oil droplets containing lipophilic drugs and ready disintegration in the gastrointestinal tract. *Pharm. Res.* **1993**, *10*, 1115−1122.

(14) Junyaprasert, V. B.; Mitrevej, A.; Sinchaipanid, N.; Boonme, P.; Wurster, D. E. Effect of process variables on the microencapsulation of vitamin A palmitate by gelatin-acacia coacervation. *Drug Dev. Ind. Pharm.* **2001**, *27*, 561−566.

(15) Rowe, R. C.; Sheskey, P. J.; Weller, P. J. *Handbook of Pharmaceutical Excipients*, 4th ed.; American Pharmaceutical Association: London, U.K., 2003; p 776.

(16) Gunning, Y. M.; Gunning, P. A.; Kemsley, E. K.; Parker, R.; Ring, S. G.; Wilson, R. H.; Blake, A. Factors affecting the release of flavor encapsulated in carbohydrate matrixes. *J. Agric. Food Chem.* **1999**, *47*, 5198−5205.

(17) Kim, Y. D.; Morr, C. V. Microencapsulation properties of gum Arabic and several food proteins: Spray-dried orange oil emulsion particles. *J. Agric. Food Chem.* **1996**, *44*, 1314−1320.

(18) Anandaraman, S.; Reineccius, G. A. Analysis of encapsulated orange peel oil. *Perfum. Flavor.* **1987**, *12*, 33−39.

(19) Lee, H. Y.; Lee, S. J.; Cheong, I. W.; Kim, J. H. Microencapsulation of fragrant oil via *in situ* polymerization: Effects of pH and melamine−formaldehyde molar ratio. *J. Microencapsulation* **2002**, *19*, 559−569.

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